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Inflammation and brain edema: new insights into the role of chemokines and their receptors

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Summary

Brain edema is associated with a variety of neuropathological conditions such as brain trauma, ischemic and hypoxic brain injury, central nervous system infection, acute attacks of multiple sclerosis, and brain tumors. A common finding is an inflammatory response, which may have a significant impact on brain edema formation. One critical event in the development of brain edema is blood-brain barrier (BBB) breakdown, which may be initiated and regulated by several proinflammatory mediators (oxidative mediators, adhesion molecules, cytokines, chemokines). These mediators not only regulate the magnitude of leukocyte extravasation into brain parenchyma, but also act directly on brain endothelial cells causing the loosening of junction complexes between endothelial cells, increasing brain endothelial barrier permeability, and causing vasogenic edema. Here we review junction structure at the BBB, the effects of pro-inflammatory mediators on that structure, and focus on the effects of chemokines at the BBB. New evidence indicates that chemokines (chemoattractant cytokines) do not merely direct leukocytes to areas of injury. They also have direct and indirect effects on the BBB leading to BBB disruption, facilitating entry of leukocytes into brain, and inducing vasogenic brain edema formation. Chemokine inhibition may be a new therapeutic target to reduce vasogenic brain edema.

Keywords: Blood-brain barrier; monocyte chemoattractant protein; CCL2; tight junctions.

Introduction

Inflammation and proinflammatory mediators play an essential role in edema development in a variety of neuropathological conditions such as brain trauma, ischemic or hypoxic brain injury, central nervous system (CNS) infection (HIV infection, tuberculosis, or bacterial meningitis), acute attacks of multiple sclerosis, and brain tumors [12, 19, 21, 29, 45, 49, 64]. Brain edema in all of these conditions is mostly classified as vasogenic with extracellular water accumulation resulting from blood-brain barrier (BBB) disruption and massive infiltration of leukocytes. The critical pathophysiological mechanism in vasogenic brain edema formation is BBB disruption, characterized by activation of brain endothelial cells followed by loosening of junctional complexes between those cells and increased barrier permeability. This is accompanied by leukocyte recruitment into the brain parenchyma and extravasation of plasma proteins [64]. We reviewed current knowledge on the ability of proinflammatory mediators to regulate BBB permeability and how they contribute to brain edema formation.

Unique properties of the BBB and cerebral endothelium

Under basal conditions, the BBB acts as a highly specialized structural and biochemical barrier that regulates the entry of blood-borne molecules into brain and preserves ionic homeostasis within the brain microenvironment [53, 67]. The BBB is composed of a specialized tight adherent microvascular endothelium and glial cell elements (astrocytes and microglia) along the entire endothelial abluminal surface [48]. Specific properties of the brain endothelial barrier are the presence of continuous strands of intercellular junction complexes that almost completely seal the paracellular cleft between adjacent endothelial membranes [4, 53]. Two morphologically distinct structural units occur in these intercellular junctional complexes: tight junctions (TJs) and adherens junctions [25].

TJs at the BBB are composed of an intricate combination of transmembrane integral proteins and several cytoplasmic-accessory proteins classified into 2 major groups: postsynaptic density (PDZ) domain containing proteins, and non-PDZ proteins [9, 25]. The major structural proteins of TJs are: (i) claudins (claudin-1, -5, -11), tissue-specific proteins that form the primary seal of TJ, (ii) occludin, an integral membrane protein involved in regulation of electrical resistance across the BBB and paracellular permeability, and (iii) junctional adhesion molecules (JAMs; JAM-1, -2, -3), single membrane-spanning proteins that belong to the immunoglobulin superfamily, which are mostly involved in leukocyte-endothelial cell interaction and leukocyte transmigration [9, 13, 25, 32, 40]. The TJ accessory proteins are multi-domain cytoplasmic molecules that form structural support for the TJ and are involved in signal transduction. The TJ PDZ-containing proteins are zonula occludens proteins (ZO-1, ZO-2, ZO-3) and AF6. The group of non-PDZ TJ proteins contains cingulin, 7H6, and atypical protein kinase C [9, 13, 25, 40].

Although the proteins of the TJ complex ultimately determine the barrier properties of endothelial cells, the adherens junction (AdJ) proteins mediate initial adhesion between endothelial cells and modulate TJ permeability [59]. The adherens junction complex is composed of a cadherin-catenin complex (Ve cadherin bound to β-catenin, plakoglobin, and α-catenin) and associated proteins (e.g., p 120 protein) [42, 59]. Both TJ and AdJ proteins are linked to cytoskeletal proteins. Actin, the primary cytoskeletal protein, has both structural and dynamic roles within cells [57, 62].

BBB opening: morphological aspects

At the functional level, the junctional complexes result in a high transendothelial electrical resistance, typically 1500–2000 $\Omega.cm^2$, making the BBB a unique selective permeability barrier [25]. Alterations in brain endothelial junctional complexes can result in increased in BBB permeability. At the cellular level, BBB "opening" is manifested by intercellular gap formation, changes in cell shape, reorganization of actin microfilament bundles, and redistribution of endothelial junction proteins. The typical morphological pattern of actin reorganization involves increased stress fiber density and a reduction or loss of the cortical actin band. This pattern occurs in parallel with and/or could cause spatial redistribution of both AdJ and TJ structures. There is decreased association of the cadherin/catenin complex with a shift to intracellular pools, disorganization of occludin on the endothelial surface with a loss of ZO-1, and increased association of occludin, ZO-1, and ZO-2 with actin filaments, which shifts these proteins to an insoluble cytosolic pool [15, 20, 63, 66]. The loss of adherence between brain endothelial cells, accompanied by conformational changes in TJ proteins and increased intercellular forces, widens the gap between brain endothelial cells and facilitates extravasation of leukocytes and plasma proteins into the brain parenchyma. Alterations in brain endothelial junctions represent a basic substrate for developing vasogenic brain edema.

Inflammation, proinflammatory mediators, and BBB opening

In conditions such as multiple sclerosis, AIDS-associated encephalopathy, and meningitis/meningoencephalitis, inflammation is considered a primary cause of brain damage [26, 39, 46]. On the other hand, in conditions such as Alzheimer's disease and stroke, inflammation can induce secondary brain injury by aggravating the initial insult [16, 22]. In all of these cases, there are the classic hallmarks of brain inflammation: BBB breakdown, edema formation, tissue infiltration by peripheral blood cells, activation of immunocompentent cells, and intrathecal release of numerous immune mediators such as interleukins and chemotactic factors [33]. Common pathological findings are the presence of many leukocytes (monocytes or neutrophils) and activated microglial cells in the brain parenchyma, and a loss of immunostaining for most TJ proteins (occludin, ZO-1, claudin-5, or ZO-2) at the BBB [6, 26, 31, 43]. This may be an indicator of TJ protein redistribution/reorganization at the junction complex.

Proinflammatory mediators play a crucial role in regulation of CNS inflammation as well as in modulating BBB permeability. For example, proinflammatory cytokines (IL-1α, IL-1β, TNF-α, IL-6, GM-CSF) produced by invading leukocytes, activated endothelial cells, or other components associated with the BBB (astrocytes or perivascular macrophages), regulate the magnitude and persistence of inflammatory brain reactions [5, 46, 68]. But those factors also directly through their own receptors, or indirectly via other mediators, participate in BBB disruption. IL-1β and TNF-α increase BBB permeability under in vitro and in vivo

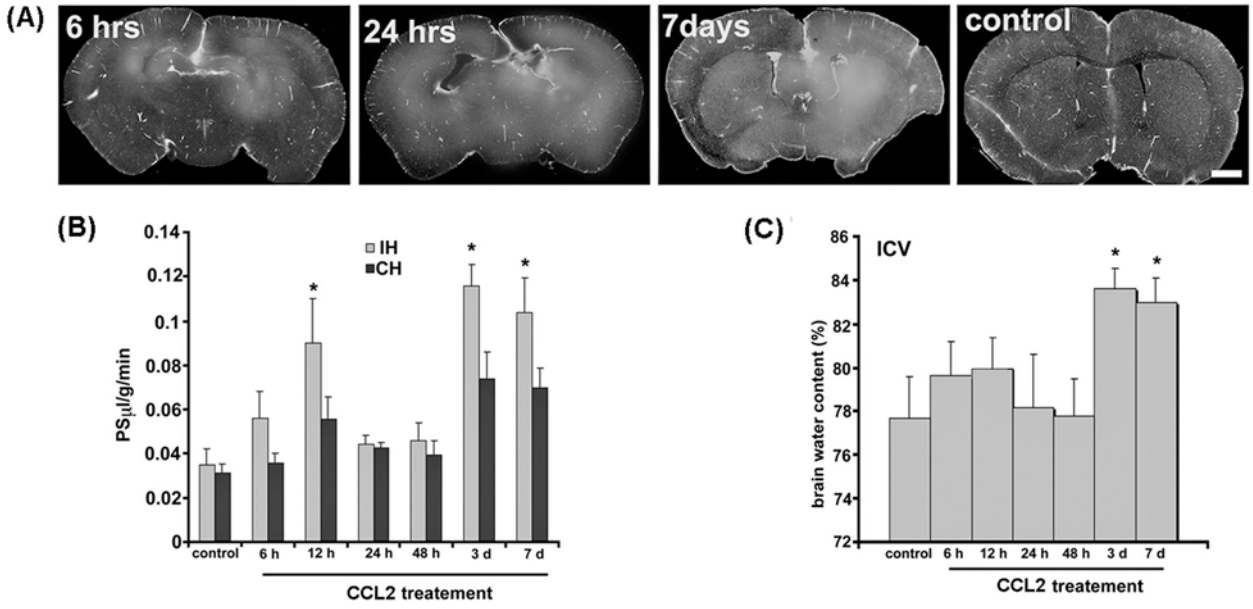

Fig. 1. Intracerebroventricular administration of CCL2 increases BBB permeability and brain edema formation. (A) Distribution of FITC-albumin in brain coronal sections; (B) permeability surface (*PS*) area products for FITC albumin; and (C) brain water content in CCL2- and saline-treated control mice. Measurements were made 6, 12, 24, and 48 hours after a single CCL2 dose (25 μg) or after a 3-day (5 μg/hr) or 7-day (2.5 μg/hr) chronic infusion. PS products were measured in ipsilateral (*IH*) and contralateral (*CH*) hemispheres. Brain water content measurements are of the whole brain. Values are mean ± SD. * indicates significant difference between CCL2-treated mice and control animals at the $p < 0.001$ level. Scale bar = 1000 μm

conditions, altering endothelial cell junction complexes leading to the development of local inflammatory responses and edema formation [5, 18, 68]. Neutralizing antibodies to these cytokines can diminish brain edema formation [24, 44].

Oxidative stress and mediators of oxidative injury (superoxide, hydrogen peroxide, peroxynitrite, nitric oxide, eicosanoids) are a second group of factors implicated in reducing TJ integrity and causing edema formation [23, 27]. Oxidative mediators can directly trigger a signal cascade with one end-point being a redistribution of TJ and AdJ proteins and reorganization of the endothelial actin cytoskeleton. Oxidative mediators can also regulate the expression/activity of other proinflammatory mediators and, in this way, ''open'' the BBB [18, 27].

Adhesion molecules (ICAM-1, VCAM-1) and selectins can also alter TJ complexes by regulating leukocyte/endothelial cell interactions. Those interactions may trigger expression of other proinflammatory mediators or intracellular signal cascades that regulate BBB integrity [14]. Leukocytes can also contribute to the persistence of increased vascular permeability via interactions with endothelial cells and the release of proinflammatory mediators, or by production of proinflammatory mediators in the brain parenchyma [11, 38, 47]. One particularly interesting group of proinflammatory mediators that contribute to alterations in BBB permeability are chemokines. In recent years, the role of these chemoattractant cytokines in CNS inflammation has emerged.

Chemokine-regulated BBB opening: role in brain edema formation

Chemokines are proinflammatory mediators involved in the selective driving of leukocytes into brain parenchyma. Enhanced perivascular chemokine expression is found in many pathological settings accompanied by inflammation, providing a chemoattractant gradient for leukocyte influx [41, 52].

Chemoattractant cytokines (chemokines) are a novel superfamily of structurally-related proinflammatory peptides (~70 to 90 amino acids) of low molecular weight (8 to 10 kDa). They have been divided into 4 classes on the basis of the positions of the first 2 conserved cysteine residues (C) and the number of intervening amino acids (X) between them (e.g., C,

CC, CXC, and CXXXC subfamilies) [41, 52, 69]. These subfamilies further exhibit functional differences. For example, CXC chemokines target mainly neutrophils, while CC chemokines primarily target monocytes/macrophages, eosinophils, and basophils, while CXC and CC chemokines invoke responses in lymphocytes [51, 69]. All chemokines mediate their effects by binding to 7-transmembrane G protein-coupled receptors related to rhodopsin. Some of the functional consequences of chemokine-receptor interactions are alterations in integrin adhesiveness, cell migration, polarization, and proliferation, as well as in gene expression [36, 51]. The discovery that parenchymal cells (astrocytes, oligodendroglial cells, and microglia in the CNS) and endothelial cells (including brain endothelial cells) express chemokine receptors significantly extended the possible functions of chemokines in inflammation and other (patho)physiological conditions [1, 2]. One of these novel functions is that chemokines can regulate BBB permeability.

The chemokine CXCL8 (IL-8) has already been shown to contribute to brain edema formation during ischemia/reperfusion injury. Thus, Matusmoto and colleagues [35] showed that adding an IL-8 neutralizing antibody significantly reduced edema formation in rabbit. In addition to IL-8, several studies examining the effect of IL-1β on BBB permeability have found a strong correlation between IL-1β-induced expression of chemokines, such as CINC1 and MIP-2 (CXCL2), and increased BBB permeability. Neutralizing antibodies to MIP2 and CINC1 can prevent BBB breakdown [3, 7].

A well-studied example of the effect of chemokines on BBB permeability is the action of monocyte chemoattractant protein-1 (MCP-1, CCL2). CCL2 belongs to the CC subfamily of chemokines and is involved in recruitment of monocytes/macrophages and activated lymphocytes into brain during neuropathological states [37]. CCL2 is highly expressed in the perivascular space and brain parenchyma during CNS inflammation, but it is also present in cerebrospinal fluid in several CNS inflammatory states (stroke, meningitis, multiple sclerosis) [8, 30, 34, 56, 58]. Expression of CCL2 receptor CCR2 was found on components of BBB (brain endothelial cells and astrocytes), suggesting that CCL2 might not only act on leukocyte recruitment but could also participate in regulation of the inflammatory response at the level of the BBB.

Our laboratory has shown that CCL2 (in μmol concentrations) in brain parenchyma or in cerebrospinal fluid can increase BBB permeability several fold (as measured by the permeability surface area product for fluorescein isothiocyanate-labeled albumin) and also induce brain edema formation (Fig. 1). In areas of BBB leakage, immunostaining for TJ proteins (occludin, ZO-1, ZO-2, claudin-5) was extensively reduced and there was intense infiltration of leukocytes [61]. There are 2 possible explanations of how CCL2 changes BBB permeability and causes vasogenic brain edema formation.

The effects of CCL2 on BBB permeability could be direct by acting on brain endothelial cells, or indirect by inducing production of other proinflammatory mediators by endothelial cells, astrocytes, or leukocytes. Evidence indicates that CCL2 acts via both mechanisms. In vitro treatment of brain endothelial cell monolayers or an in vitro model of BBB (co-culture of brain endothelial cells and astrocytes) with recombinant CCL2 exerts the same morphological and biochemical changes. Thus, TJ proteins are redistributed from a TritonX-100 soluble to a TritonX-100 insoluble fraction (potentially reflecting internalization of TJ proteins into a cytoplasmic compartment) and there is a loss or fragmentation of TJ protein staining [60, 61]. These biochemical and morphological alterations are absent if the CCL2 receptor CCR2 is not present on the brain endothelial cells [60, 61]. The effects of CCL2 on BBB permeability in vivo are abolished if the CCR2 receptor is deleted, pinpointing that CCL2 alters BBB permeability directly via its own CCR2 receptor.

Indirect effects of CCL2 on BBB permeability may involve regulation of leukocyte recruitment and/or regulation of the expression of proinflammatory mediators. We have found that depletion of circulating monocytes and activated macrophages by liposomally-encapsulated clodronate attenuates the effect of CCL2 on BBB permeability in vivo [61]. This implies that leukocytes are one of the factors contributing to BBB breakdown. Other factors may include proinflammatory factors such as VEGF and IL-1, which are significantly reduced in CCL2 knockout mice undergoing middle cerebral artery occlusion [28].

These results on CCL2, which may also apply to other chemokines, indicate a potential key role in mediating BBB breakdown during inflammation. CCL2 may not only direct leukocytes to sites of injury, but it may facilitate leukocyte migration into brain parenchyma by enhancing BBB permeability. Such BBB breakdown may also result in vasogenic edema.

Molecular mechanisms underlying regulation of permeability by CCL2

In general, several signal pathways regulate BBB permeability. For alterations in actin cytoskeleton architecture, prominent roles are proposed for: (i) Ca^{2+}/calmodulin- and Rho/Rho kinase-dependent pathways, primarily acting on the activity of myosin light chain kinase in order to facilitate actin and myosin light chain interaction and stress fiber formation. (ii) Phosphorylation of TJ and AdJ proteins by protein kinase C isoforms, tyrosine kinase lyn, serine kinase, and protein tyrosine phosphatase. (iii) Under some circumstances, proteolytic degradation of junctional constituents by matrix metalloproteinases (MMP-2, MMP-9) leading to the loss of TJs and AdJs and increased BBB permeability [10, 17, 18, 20, 50, 54, 65].

Chemokines, like other proinflammatory mediators, have a prolonged effect on the brain endothelial barrier denoted as *thrombin type* and characterized by intercellular widening for more than 30 minutes and specific activation of Rho/Rho kinase pathways. Two independent studies on 2 different types of model systems have shown that chemokines CXCL8 and CCL2 induce activation of Rho/Rho kinase with stress fiber formation [55, 60]. Further, Stamatovic *et al.* [60] found that Rho/Rho kinase not only altered the actin cytoskeletal organization but also had a significant impact on TJ protein complexes between brain endothelial cells. Diminishing Rho activity by transfection with dominant negative mutant of Rho stabilized brain endothelial barrier integrity. This led to the conclusion that Rho is a ''nodal point'' in brain endothelial intercellular signaling directed to alter junction complex of brain endothelial cells. Future investigation is needed to elucidate how Rho induces the phosphorylation and redistribution of TJ proteins.

Conclusion

Proinflammatory mediators, including chemokines, have a significant impact on brain edema formation in a variety of neuropathological conditions. Experiments examining CCL2, studied as a prototype for chemokine activity, show effects on leukocyte extravasation not only through directing leukocytes into the brain parenchyma but also by enhancing BBB disruption. CCL2 affects brain endothelial cells directly (via CCR2 receptors) and indirectly (via production of other proinflammatory agents). CCL2-induced BBB disruption may contribute to plasma protein extravasation and vasogenic brain edema as well as aggravating leukocyte influx. Chemokines are emerging as powerful factors controlling inflammation-induced brain edema. Elimination of chemokine activity via newly developed chemokine receptor antagonists offers a new potential avenue for the treatment of vasogenic brain edema.

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